Version 27-07/24

User's Manual

Diazepam ELISA Kit

REF **DEIA053NS**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is a competitive enzyme immunoassay for the quantitative analysis of Diazepam in Muscle,Urine,Feed. Please contact your area sales manager for the applicability if other sample need to be tested.

Principles of Testing

This kit uses Competitive-ELISA as the method. It can detect Diazepam (DZP) in samples, such as muscle, feed. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen During the reaction, DZP in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-DZP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of DZP. The concentration of DZP in the samples can be calculated by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

1. ELISA Microtiter plate, 96 wells
2. Standard Liquid, 1mL each,(ppb=ng/mL=ng/g, 0ppb, 0.3 ppb,0.9ppb, 2.7ppb, 8.1ppb, 24.3 ppb)
3. HRP Conjugate, 11 mL
4. Antibody Working Solution, 5.5 mL
5. Substrate Reagent A, 6 mL
6. Substrate Reagent B, 6 mL
7. Stop Solution, 6 mL
8. 20×Concentrated Wash Buffer, 40 mL
9. 2×Reconstitution Buffer, 50 mL
10. Plate Sealer, 3 pieces
11. Sealed Bag, 1 piece
12. Manual, 1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution

Materials Required But Not Supplied

Instruments: Microplate reader, Printer, Homogenizer, Vortex mixer, Nitrogen evaporators, Water bath, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g)

Micropipette: Single channel (20-200 uL, 100-1000 µL), Multichannel (30-300 uL).

Reagents: NaOH, N-hexane.

Storage

Store the kit at 2-8°C. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C.

Expiry date: expiration date is on the packing box.

Specimen Collection And Preparation

1. Pretreatment of muscle (livestock) sample

a. Remove fat from sample, homogenize the sample with homogenizer.

b. Weigh 2±0.05 g of homogenate muscle sample, add 8 mL of 0.1 M NaOH Solution (Solution 3). Vortex fully for 5 min, centrifuge at 4000 r/min for 10 min at room temperature.

c. Take 1 mL of the supernatant, add 10 mL of N-hexane. Vortex fully for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.

d. Take 5 mL of the upper N-hexane phase to glass tube and dry at 50-60°C with nitrogen evaporators or water bath.

e. Take 1 mL of the Reconstitution Buffer (Solution 1) to redissolve dry material.

f. Take 50 uL for analysis.

Note: Sample dilution factor: 10, detection limit: 5 ppb

2. Pretreatment of urine (swine) sample:

a. Take 1 mL of clear urine sample into 50 mL centrifuge tube. Add 4 mL of 0.1 M NaOH Solution (Solution 3). Vortex fully for 2 min

b. Take I mL of the mixture, add 10 mL of N-hexane. Vortex fully for 5 min, centrifuge at 4000 r/min for 5 min at room temperature.

c. Take 5 mL of the upper N-hexane phase and to glass tube and dry at 50-60°C with nitrogen evaporators or water bath.

d. Take 1 mL of the Reconstitution Buffer (Solution 1) to redissolve dry material.

e. Take 50 uL for analysis.

Note: Sample dilution factor: 10, detection limit: 5 ppb.

3. Pretreatment of feed sample:

a. Homogenize the representative sample with a homogenizer and mix fully.

b. Weigh 1±0.05 g of homogenate feed sample, add 1 mL of deionized water and 3 mL of 0.1 M NaOH Solution (Solution 3). Vortex fully for 2 min.

c. Add 10 mL of N-hexane. Vortex fully for 10 min, centrifuge at 4000 r/min for 10 min at room temperature.

d. Take 1 mL of the upper N-hexane phase and to glass tube and dry at 50-60°C with nitrogen evaporators or water bath,

d. Take I mL of the Reconstitution Buffer (Solution 1) to redissolve dry material. Then dilute it with the following ratio.

e. For formula feed sample: Dilute the sample solution [Step c] with Reconstitution Buffer (Solution 1) for 10 times (sample solution: Reconstitution buffer = 1:9).

Note: Sample dilution factor: 100, detection limit: 50 ppb

f. For other feed sample: Dilute the sample solution [Step c] with Reconstitution Buffer (Solution 1) for 20 times (sample solution: Reconstitution bufer = 1:19)

Note: Sample dilution factor: 200, detection limit: 100 ppb

Reagent Preparation

Restore all reagents and samples to room temperature before use. Open the microplate reader in advance, precheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clcan, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation:

Please prepare solution according to the nmumber of samples. Don't use up all components in the kit at once!

Solution 1: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer: Deionized water-1:1) .The Reconstitution buffer can be store at 4C for a month.

Solution 2: Wash Buffer

Dilute the 20× Concentrated Wash Buffer with deionized water 20× Concentrated Wash Buffer: Deionized water=1:19)

Solution 3: 0.1 M NaOH Solution

Dissolve 4 g of NaOH to 1000 mL with deionized water.

Assay Procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. Number: number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Standard and Samples need test in duplicate.
2. Add Sample: add 50 uL of Standard or Sample per well, then add 5o uL Antibody Working Solution, cover the plate with plate sealer. Oscillate for 5s gently to mix thoroughly. Incubate at 25°C for 30 min in shading light.
3. Wash: uncover the sealer carefully, remove the liquid in cach well. Immediately add 300 ul of Wash Buffer (Solution 2) to cach well and wash. Repeat wash procedure for 5 times, 30s intervals/time. Invert the plate

and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).

4. HRP Conjugate: add 100 uL of HRP Conjugate to each well. Incubate at 25°C for 30 min in shading light.
5. Wash: Repeat Step 3.
6. Color Development: add 50 uL of Substrate Reagent A to cach well, and thcn add 50 uL of Substrate Reagent B.Gently oscillate for 5s to mix thoroughly. Incubate at 25°C for 15 min in shading light.
7. Stop Reaction: add 50 uL of Stop Solution to each well, gently oscillate for 5s
8. OD Measurement: determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min after stop reaction.

Calculation

1. Absorbance%=A/A_0×100%

A: Average absorbance of standard solution or sample

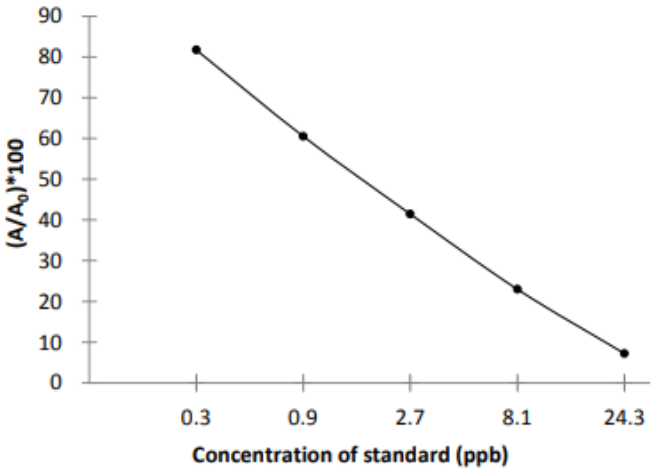
A_0: Average absorbance of 0 ppb Standard solution

2. Drawing and caleulation of standard curve

Create a standard curve by plotting the absorbance percentage of cach standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on many samples.

Typical Standard Curve



Specificity

Cross-reactivity:

Diazepam ---100%,

Nitrazepam---<10%,

Oxazepam---<10%.

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